a' Cont

20. (Amended) The enriched or purified preparation of oligodendrocyte progenitor cells of claim 19 which are human.

REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully traversed. Pursuant to 37 CFR § 1.21, attached as an Appendix is a Version of Amended Claims With Markings to Show Changes Made.

The damaged brain is largely incapable of functionally significant structural self-repair. This is due in part to the apparent failure of the mature brain to generate new neurons and oligodendrocytes. Converging lines of evidence now support the contention that neuronal and glial precursor cells are distributed widely throughout the adult vertebrate forebrain, persisting across a wide range of species groups. Most studies have found that the principal source of these precursors is the ventricular zone, though competent neural precursors have been obtained from parenchymal sites as well. In general, adult progenitors respond to epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) with proliferative expansion, may be multipotential, and persist throughout life. In rodents and humans, their neuronal daughter cells can be supported by brain-derived neurotrophic factor (BDNF) and become fully functional *in vitro*, like their avian counterparts.

A major impediment to both the analysis of the biology of adult neural precursors, and to their use in engraftment and transplantation studies, has been their relative scarcity in adult brain tissue and their consequent low yield when harvested by enzymatic dissociation and purification techniques. As a result, attempts at either manipulating single adult-derived precursors or enriching them for therapeutic replacement have been difficult. The few reported successes at harvesting these cells from dissociates of adult brain, whether using avian, murine, or human tissue, have all reported <1% cell survival. Thus, several groups have taken the approach of raising lines derived from single isolated precursors, continuously exposed to mitogens in serum-free suspension culture. As a result, however, many of the basic studies of differentiation and growth control in the neural precursor population have been based upon small numbers of founder cells, passaged greatly over prolonged periods of time, under constant mitogenic stimulation. The phenotypic potential, transformation state and karyotype of these cells are all uncertain; after repetitive passage, it

is unclear whether such precursor lines remain biologically representative of their parental precursors, or instead become transformants with perturbed growth and lineage control.

In order to devise a more efficient means of isolating native, unpassaged and untransformed progenitor cells from brain tissue, a strategy by which brain cells could be freely dissociated from brain tissue, then transduced *in vitro* with plasmid DNA bearing a fluorescent reporter gene under the control of neural progenitor cell-type specific promoters, was developed. This permitted live cell identification of the elusive neuronal progenitor cell of the CNS, using the Tα1 tubulin promoter, a regulatory sequence expressed only in neuronal progenitor cells and young neurons, and the isolation and purification of this cell type using FACS (i.e. fluorescence activated cell sorting).

The repair of damaged brain requires not only sources of new neurons but also new glial support cells. Oligodendrocytes are the glial cell type that produce myelin and insulate neuronal axons by ensheathment with myelin-bearing cell processes. Like neurons, human oligodendrocytes are largely postmitotic and cannot regenerate through proliferative expansion. However, persistent oligodendrocyte progenitors have been described in adult rodent subcortical white matter and may provide a substrate for remyelination after demyelinating injury. In humans, the demonstration and identification of analogous subcortical oligodendrocyte progenitor cells had been problematic. A pre-oligodendrocytic phenotype has been described in adult human subcortical white matter, though these postmitotic cells may have included mature oligodendrocytes recapitulating their developmental program after dissociation. Rare examples of oligodendrocytes derived from mitotic division have also been reported in human subcortical dissociates, but the identification and isolation of their mitotic progenitors have proven elusive. As a result, the enrichment of these cells for functional utilization has proven difficult. In particular, the cells have not been preparable in the numbers or purity required for *in vivo* engraftment into demyelinated recipient brain, whether experimentally or for clinical therapeutic purposes.

A strong need therefore exists for a new strategy for identifying, separating, isolating, and purifying native oligodendrocyte precursor cells from brain tissue. Such isolated, enriched native precursors may be used in engraftment and transplantation in demyelinating disorders, as well as for studies of their growth control and functional integration.

The present invention is directed to overcoming the deficiencies in the art.

With regard to the Information Disclosure Statement filed on March 31, 2000, applicants are submitting with this response a new PTO-1449 form listing the references which were deleted, in the outstanding office action, from the prior version of this form. Copies of the references listed in the attached form are also enclosed. It is respectfully requested that these references be considered and that the enclosed PTO-1449 Form be initialed by the examiner and returned with the next written communication from the U.S. Patent and Trademark Office to reflect such consideration.

The rejection of claims 17-20 under 35 U.S.C. § 102(a) as anticipated by Wang, et. al., "Isolation and Purification of Oligondendrocyte Progenitor Cells from the Adult Human Subcortex," Ann. Neurol. 44(3): 438 (September 1998)("Wang Abstract") is respectfully traversed.

The enclosed Declaration of Steven A. Goldman Under 37 C.F.R. § 1.132 ("Goldman Declaration") demonstrates that only Dr. Goldman and his co-inventor, Su Wang, invented the subject matter of the present application. See In re Katz, 687 F.2d 450, 215 U.S.P.Q. 14 (CCPA 1982). In particular, these inventors alone conceived of an enriched or purified preparation of mitotic oligodendrocyte progenitor cells, oligodendrocytes generated from such mitotic progenitor cells, and a process for obtaining them from the human central nervous system (Goldman Declaration ¶ 4). Catherine Harrison-Restelli was a technician in Dr. Goldman's laboratory who dissected tissue, and prepared culture medium and plates at the instruction of the named inventors (Id. at ¶ 5). Afzal Naiyer was a junior post-doctoral fellow who assisted Ms. Harrison-Restelli in her responsibilities as part of his education and training (Id. at ¶ 6). Richard Fraser provided tissue samples to the named inventors, at their request, in order for Drs. Goldman and Wang to carry out their experimental work (Id. at ¶ 7). Michel Gravel and Peter Braun provided CNP promoter constructs to the named inventors, at the named inventors' request, to carry out their experimental work (Id. at ¶ 8). Since only applicants, Steven A. Goldman and Su Wang, invented the claimed invention, the subject matter of the Wang abstract was not invented by "another". Accordingly, the rejection under 35 U.S.C. § 102(a) based on this reference should be withdrawn.

The rejection of claims 17-20 under 35 U.S.C. § 102(b) over Armstrong, et. al., "Pre-Oligodendrocytes from Adult Human CNS," J. Neurosci. 12(4): 1538-47 (1992) ("Armstrong") is respectfully traversed.

Armstrong discloses culturing white matter from human patients undergoing partial temporal lobe resection for intratable epilepsy to determine whether oligodendrocyte precursor cells are present in the adult human central nervous system. These cultures contained cells that expressed antigens recognized by the O4 monoclonal antibody, indicating that they were of oligodendrocyte lineage. It was theorized that O4-positive cells appear to develop into oligodendrocytes, so they were referred to as pre-oligodendrocytes. However, these pre-oligodendrocytes did not divide in response to growth factors that typically trigger mitosis of oligodendrocyte progenitors. Since the pre-oligodendrocytes of Armstrong and the oligodendrocytes they produce did not undergo cell division (i.e. mitosis) when treated with mitosis-triggering agents, it is clear that these cells are not mitotic. This was recognized explicitly by Armstrong, with the study leading to the present invention being undertaken specifically to address this critical failure of Armstrong. By contrast, the oligondendrocyte progenitor cells of the present invention are clearly mitotic and give rise to new oligodendrocytes as indicated by the following passage on page 22 of the present application:

> These data indicate that the adult human subcortex harbors a population of residual, mitotically-competent oligodendrocyte progenitor cells. The cells constitute a discrete population of bipolar blasts, distinct from mature oligodendrocytes. The progenitors were mitotically competent, and as such, distinct from the much larger population of mature, apparently post-mitotic oligodendrocytes. These cells were antigenically immature ($A2B5^+/O4^-$) when isolated, but matured ($O4^+/O1^+$) over several weeks in culture. Cell-specific targeted reporting, achieved by transfecting the overall white matter pool with plasmids of GFP placed under the control of the early promoter for oligodendrocytic CNP, allowed the live-cell identification of these progenitor cells. This in turn provided a means for their isolation and purification, by fluorescence-activated cell sorting based on P/hCNP2-driven GFP expression.

Since Armstrong does not teach mitotic oligodentrocyte progenitor cells, it cannot be used as a proper basis to reject the claims. Accordingly, the rejection based on this reference should be withdrawn.

The rejection of claims 17-20 under 35 U.S.C. § 102(b) over Kirschenbaum, et. al., "*In Vitro* Neuronal Production and Differentiation by Precuror Cells Derived from the

Adult Human Forebrain," Cerebral Cortex 6: 576-89 (1994) ("Kirschenbaum") is respectfully traversed.

Kirschenbaum cultures samples of adult temporal lobes under conditions suitable for neuronal differentiation, while exposed to ^{3}H-thymidine. These samples were incubated for 7-28 days, stained for neuronal and glial antigens, and autoradiographed. Neuron-like cells were found in explant outgrowths and mononlayer dissociates of the subependymal zone and periventricular white matter but not the cortex. A small number of Map-2^{+} and Map-5^{+}/glial fibrillary acidic protein^{-} cells did incorporate ^{3}H-thymidine, suggesting neuronal production from precursor mitosis. However, the O4^{+} oligondendrocytes were postmitotic. The study described in Kirschenbaum was carried out in the laboratory of applicant Steven A. Goldman, M.D., Ph.D and caused applicants to address the failure of the Kirschenbaum study to identify mitotic oligodendrocyte progenitor cells. This led to the present invention. Since Kirschenbaum does not disclose mitotic oligodendrocyte progenitor cells or oligodendrocytes generated from such progenitors as claimed, the rejection based on this reference should be withdrawn.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: June 4, 2001

Michael L. Goldman
Registration No. 30,727

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603
Telephone: (716) 263-1304
Facsimile: (716) 263-1600

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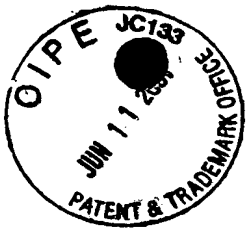

Appendix A

Version of Amended Claims With Markings to Show Changes Made

In reference to the amendments made herein to claims 17 and 19, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In the Claims:

17. (Amended) An enriched or purified preparation of [isolated], postnatal oligodendrocytes, generated from mitotic progenitor cells.

18. (Amended) The enriched or purified preparation of [isolated] oligodendrendrocytes of claim 17 which are human.

19. (Amended) An enriched or purified preparation of isolated, mitotic oligodendrocyte progenitor cells.

20. (Amended) The enriched or purified preparation of [isolated] oligodendrocyte progenitor cells of claim 19 which are human.